FORM PTO-1390 U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371	ATTORNEY'S DOCKET NUMBER: USB 98 AX CNR NFK U.S. APPLICATION NO. (If known, see 37 CFR 1.5) 09/856796

INTERNATIONAL APPLICATION NO.:	INTERNATIONAL FILING DATE:	PRIORITY DATE CLAIMED:
PCT/FR99/02897	24 November 1999	25 November 1998

TITLE OF INVENTION: NF-κB ACTIVATION INHIBITORS, AND THEIR PHARMACEUTICAL USES

APPLICANT(S) FOR DO/EO/US: François HIRSCH, Astrid HAEFFNER

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. [X] This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. [ ] This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. [X] This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. [X] A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. [X] A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. [X] is transmitted herewith (required only if not transmitted by the International Bureau).
   b. [ ] has been transmitted by the International Bureau. (see attached copy of PCT/IB/308)
   c. [ ] is not required, as the application was filed in the United States Receiving Office (RO/US).
6. [X] A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. [ ] Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)).
   a. [ ] are transmitted herewith (required only if not transmitted by the International Bureau).
   b. [ ] have been transmitted by the International Bureau.
   c. [ ] have not been made; however, the time limit for making such amendments has NOT expired.
   d. [ ] have not been made and will not be made.
8. [ ] A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. [ ] An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. [ ] A translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Item 11. to 16. below concern document(s) or information included:

11. [X] An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. [ ] An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. [X] A **FIRST** preliminary amendment.
    [ ] A **SECOND** or **SUBSEQUENT** preliminary amendment.
14. [ ] A substitute specification.
15. [ ] A change of power of attorney and/or address letter.
16. [X] Other items or information:

International Preliminary Examination Report (PCT/IPEA/409)
International Search Report (PCT/ISA/210)
French Search Report
Abstract on a separate sheet
Sequence listing on paper and on diskette in computer-readable form
Application Data Sheet

531 Rec'd PCT 25 MAY 2001

U.S. APPLICATION NO. (if known, see 37 CFR 1.5)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NO.
09/856796	PCT/FR99/02897	USB 98 AX CNR NFK

	CALCULATIONS PTO USE ONLY
17. [X] The following fees are submitted:	
BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(5)):	
Neither international preliminary examination fee (37 CFR1.482) nor international search fee (37 CFR1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO ..... $ 1,000.00	
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO ..... $ 860.00	
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO ..... $ 710.00	
International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) ..... $ 690.00	
International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) ..... $ 100.00	
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 860.00
Surcharge of $130.00 for furnishing the oath or declaration later than 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$ 130.00

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	
Total claims	12 - 20 =	0	X $18.00	$
Independent claims	2 - 3 =	0	X $80.00	$
MULTIPLE DEPENDENT CLAIMS(S) (if applicable)			+ $270.00	$
			TOTAL OF ABOVE CALCULATIONS =	$ 990.00
Reduction of ½ for filing by small entity, if applicable. Applicant claims Small Entity Status under 37 CFR 1.27. +				$
			SUBTOTAL =	$ 990.00
Processing fee of $130 for furnishing the English translation later than months from the earliest claimed priority date (37 CFR1.49(f)).				$
			TOTAL NATIONAL FEE =	$ 990.00
Fee for recording the enclosed assignment (37 CFR1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). $40.00 per property +				$
			TOTAL FEES ENCLOSED =	$ 990.00
			Amount to be refunded:	
			charged:	

a. [X] A check in the amount of $ **990.00** to cover the above fees is enclosed.

b. [ ] Please charge my Deposit Account No. **25-0120** in the amount of $ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. [X] The Commissioner is hereby authorized to charge any additional fees which may be required by 37 CFR 1.16 and 1.17, or credit any overpayment to Deposit Account No. **25-0120**. A duplicate copy of this sheet is enclosed.

SEND ALL CORRESPONDENCE TO:

Customer No. 000466
YOUNG & THOMPSON
745 South 23rd Street
2nd Floor
Arlington, VA 22202
(703) 521-2297 facsimile (703) 685-0573

May 25, 2001

By _____
Andrew J. Patch
Attorney for Applicants
Registration No. 32,925

PATENTS

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of

François HIRSCH et al.

Box PCT

Serial No. (unknown)

Application Branch

Filed herewith

NF-κB ACTIVATION INHIBITORS, AND THEIR PHARMACEUTICAL USES

PRELIMINARY AMENDMENT

Commissioner for Patents

Washington, D.C. 20231

Sir:

Prior to the first Official Action and calculation of the filing fee, please amend the above-identified application as follows:

IN THE CLAIMS:

Amend claim 3 as follows:

--3. (amended) The use according to claim 1, of compounds inhibiting the activation of MF-κB connected specifically to the transmembranal receptors of the cytokins of class I in the cells of the organism, such as compounds selected from growth hormone or erythropoietin.--

Amend claim 4 as follows:

--4. (amended) The use according to claim 1:

- of the human growth hormone, as obtained by extraction from hypophysary extracts, and purification,

Amend claim 6 as follows:

--6. (amended) The use compounds inhibiting the activation of NF-κB according to claim 1, in combination with one or several cytotoxic molecules adapted to activate the NF-κB factor, selected from:

- cytokines,
- anthracyclines, including daunomycin, and dauxorubicin,
- vinca-alkaloids, such as vinblastine and vincristine,
- paclitaxel (or Taxel, DCI).--

Amend claim 7 as follows:

--7. (amended) The use of compounds inhibiting the activation of NF-κB according to claim 1, characterized in that the dosage of the cytotoxic molecules used in combination with said compounds is about 2 to about 5 times less than the dosage of these same molecules used alone in the scope of treatment of malignant hemopathies and solid tumors.--

--10. (amended) Product according to claim 8, characterized in that it comprises:

- human growth hormone, such as obtained by the extraction from hypophysary extracts, and purification,
- or recombinant human growth hormone as encoded by the nucleotide sequence SEQ ID NO 1, or by any nucleotide sequence derived from this latter by degeneracy of the genetic code and being nevertheless capable of encoding for human growth hormone whose amino acid sequence is represented by SEQ ID NO 2, said growth hormone being obtained by a transformation of suitable cells with the help of vectors containing a

nucleotide sequence such as described above, recovery of the recombinant protein produced by said cells, and purification,

- or any peptide sequence derived by addition and/or deletion and/or substitution of one or several amino acids of the sequence SEQ ID NO 2, and keeping the property of the human growth hormone of inhibiting the activation of NF-κB.--

Amend claim 11 as follows:

--11. (amended) Product according to claim 8, characterized in that it comprises:

- recombinant human erythropoietin as encoded by the nucleotide sequence SEQ ID NO 3, or by any nucleotide sequence derived from this latter by degeneracy of the genetic code and being nevertheless capable of encoding for human erythropoietin whose sequence in amino acids is represented by SEQ ID NO 4, said erythropoietin being obtained by transformation of suitable cells with the help of vectors containing a nucleotide sequence as described above, recovery of the recombinant protein produced by said cells, and purification,

- or any peptide sequence derived by addition and/or deletion and/or substitution of one or several amino acids of the sequence SEQ ID NO 4, and keeping the property of human erythropoietin of inhibiting the activation of NF-κB.--

Amend claim 12 as follows:

--12. (amended) Product according to claim 8, characterized in that it comprises as cytotoxic molecule susceptible of activating the NF-κB factor, any molecule selected from the following:

- cytokines,
- anthracyclines including daunomycin and dauxorubicin,
- vinca-alkaloids, including vinblastine and vincristine,
- paclitaxel (or Taxol, DCI).---

R E M A R K S

The above changes in the specification and claims merely place this national stage application in the same condition as it was during Chapter II of the international stage, with the multiple dependencies being removed.

Attached hereto is a marked-up version of the changes made to the claims by the current amendment. The attached page is captioned "VERSION WITH MARKINGS TO SHOW CHANGES MADE."

Respectfully submitted,

YOUNG & THOMPSON

By

Andrew J. Patch
Attorney for Applicants
Registration No. 32,925
Customer No. 00466
745 South 23rd Street
Arlington, VA 22202
Telephone: 703/521-2297

May 25, 2001

VERSION WITH MARKINGS TO SHOW CHANGES MADE

Amend claim 3 as follows:

--3. (amended) The use according to claim 1~~ or claim 2~~, of compounds inhibiting the activation of MF-κB connected specifically to the transmembranal receptors of the cytokins of class I in the cells of the organism, such as compounds selected from growth hormone or erythropoietin.--

Amend claim 4 as follows:

--4. (amended) The use according to ~~one of~~ claims ~~1 to 3~~:

- of the human growth hormone, as obtained by extraction from hypophysary extracts, and purification,

- or of the recombinant human growth hormone as encoded by the nucleotide sequence SEQ ID NO 1, or by any nucleotide sequence derived from this latter by degeneracy of the genetic code and being nevertheless capable of encoding for the human growth hormone whose sequence in amino acids is represented by SEQ ID NO 2, said growth hormone being obtained by transformation of appropriate cells with the help of vectors containing a nucleotide sequence as described, recovery of the recombinant protein produced by said cells, and purification,

- or of any peptide sequence derived by addition and/or deletion and/or substitution of one or several amino acids of the sequence SEQ ID NO 2,

and preserving the property of human growth hormone of inhibiting the activation of NF-κB.--

Amend claim 5 as follows:

--5. (amended) The use according to ~~one of~~ claim~~s~~ ~~1 to 3~~:

- of recombinant human erythropoietin such as encoded by the nucleotide sequence SEQ ID NO 3, or by any nucleotide sequence derived from this latter by degeneracy of the genetic code and being nevertheless capable of encoding for human erythropoietin whose sequence in amino acids is represented by SEQ ID NO 4, said erythropoietin being obtained by transformation of appropriate cells with the help of vectors contained in a nucleotide sequence as described above, recovery of the recombinant protein produced by said cells, and purification,

- or any peptide sequence derived by addition and/or deletion and/or substitution of one or several amino acids of the sequence SEQ ID NO 4, and preserving the property of inhibiting the activation of NF-κB.--

Amend claim 6 as follows:

--6. (amended) The use compounds inhibiting the activation of NF-κB according to ~~one of~~ claim~~s~~ ~~1 to 7~~, in combination with one or several cytotoxic molecules adapted to activate the NF-κB factor, selected from:

- cytokines,
- anthracyclines, including daunomycin, and dauxorubicin,
- vinca-alkaloids, such as vinblastine and vincristine,
- paclitaxel (or Taxel, DCI).--

Amend claim 7 as follows:

--7. (amended) The use of compounds inhibiting the activation of NF-κB according to ~~one of~~ claim~~s~~ ~~1 6~~, characterized in that the dosage of the cytotoxic molecules used in combination with said compounds is about

2 to about 5 times less than the dosage of these same molecules used alone in the scope of treatment of malignant hemopathies and solid tumors.--

--10. (amended) Product according to claim 8~~ or 9~~, characterized in that it comprises:

- human growth hormone, such as obtained by the extraction from hypophysary extracts, and purification,

- or recombinant human growth hormone as encoded by the nucleotide sequence SEQ ID NO 1, or by any nucleotide sequence derived from this latter by degeneracy of the genetic code and being nevertheless capable of encoding for human growth hormone whose amino acid sequence is represented by SEQ ID NO 2, said growth hormone being obtained by a transformation of suitable cells with the help of vectors containing a nucleotide sequence such as described above, recovery of the recombinant protein produced by said cells, and purification,

- or any peptide sequence derived by addition and/or deletion and/or substitution of one or several amino acids of the sequence SEQ ID NO 2, and keeping the property of the human growth hormone of inhibiting the activation of NF-κB.--

Amend claim 11 as follows:

--11. (amended) Product according to claim 8~~ or 9~~, characterized in that it comprises:

- recombinant human erythropoietin as encoded by the nucleotide sequence SEQ ID NO 3, or by any nucleotide sequence derived from this latter by degeneracy of the genetic code and being nevertheless capable of encoding for human erythropoietin whose sequence in amino acids is

represented by SEQ ID NO 4, said erythropoietin being obtained by transformation of suitable cells with the help of vectors containing a nucleotide sequence as described above, recovery of the recombinant protein produced by said cells, and purification,

- or any peptide sequence derived by addition and/or deletion and/or substitution of one or several amino acids of the sequence SEQ ID NO 4, and keeping the property of human erythropoietin of inhibiting the activation of NF-κB.--

Amend claim 12 as follows:

--12. (amended) Product according to ~~one of~~ claim~~s~~ ~~8 to 11~~, characterized in that it comprises as cytotoxic molecule susceptible of activating the NF-κB factor, any molecule selected from the following:

- cytokines,
- anthracyclines including daunomycin and dauxorubicin,
- vinca-alkaloids, including vinblastine and vincristine,
- paclitaxel (or Taxol, DCI).--

NF-κB ACTIVATION INHIBITORS, AND THEIR PHARMACEUTICAL USES

The present invention has for its object the use of biological inhibitors of NF-κB, in the field of treating cancers, and more particularly malignant hemopathies or solid tumors.

Numerous tumoral cells have developed sophisticated mechanisms permitting them to resist the effect of certain agents used in anti-cancer chemotherapy. One of the countermeasures at present developed by clinicians is the increase of the dosage of these medications, with the result of aggravating the side effects observed in the patients. Thus, for example, most of the leukemias and certain lymphomas are treated by the administration of anthracyclines (daunomycin, dauxorubicin) whose toxicity is manifest in the vital functions (hepatic, cardiac...) (Gauthier, PH, 1987, Gas Med Fr, 94:43-49).

The mechanism of action of the medications has been well studied and has essentially led to the death of tumor cells by apoptosis (Hannum YA, Blood, 89:1845-1853). To avoid apoptosis, the cells use a category of proteins encoded by genes called *multidrug resistant genes* (MDR) which permit them to control the intake or outflow of various molecules (Pastan I, Gottesman MM, 1991, Annu Rev Med, 42:277-286). In

the case of anti-cancer agents, these are actively evacuated by means of P-glycoprotein (P-gp), produced by the *MDR1* gene.

As all genes, the expression of the *MDRs* is controlled by different nuclear factors. Thus, it has been recently shown that the *MDR1* gene has in its regulatory portion binding sites of the NF-κB factor (Zhou G, Kuo MT, 1997, J Biol Chem, 272:15174-15183). This nuclear factor, which moreover plays a considerable role in numerous inflammatory situations (Barnes PJ, Karin M, 1997, N Engl J Med, 336:1066-1071) participates in the activation of the *MDR1* gene.

Several recent works have established a connection between the inhibition and the activation of NF-κB and the potentialization of apoptosis. In the first reported experiments (Wang CY et coll., 1996, Science, 272:784-786, Van Antwerp DJ et coll., Science, 272:787-789) the authors have validated their data by using genetically manipulated lines to obtain the inhibition or the overexpression of NF-κB activity. Thus, this does not permit their direct use in therapeutic applications.

In another study, the authors have tested the effects of different protease inhibitors preventing the activation of NF-κB (pyrolidine dithiocarbamate, *N*-tosyl-L-lysl chloromethylcetone, *N*-acetyl cysteine) on a line of murine macrophages (Mannick EE et coll., 1997, Mediators of Inflammation, 6:225-232). The authors of this article conclude there is a possible connection between NF-κB inhibition and the induction of apoptosis of the inflammatory and immune cells.

Finally, another approach based on inhibition of the

inflammatory effects of NF-κB, consists in overexpressing the natural inhibitor of NF-κB, the IκB molecule, by gene therapy (Makarov SS et coll., 1997, Gene Ther, 4:846-852). This technology is also in the state of development because of the complexity and the vectorization necessary for its good operation.

The present invention results from the discovery by the inventors of new effects of the human growth hormone (HGH), also called somatotropin, namely, on the one hand that HGH, and other compounds connected specifically to the transmembrane receptors of class I cytokines, are inhibitors of the activation of NF-κB by a cytotoxic molecule, and, on the other hand, that HGH, and other above-mentioned compounds, permit potentiating the effects of cytotoxic molecules and hence reducing the concentrations of these latter in the field of therapeutic treatments.

First of all, the inventors have observed that the human monocytes respond less to a stimulation by lipopolysaccharides (LPS) when they are cultivated in the presence of exogenous recombinant HGH. The inventors have accordingly concluded that HGH inhibits the activation of NF-κB after stimulation by LPS (Haeffner A et coll., 1997, J Immunol, 158:1310-1314).

Then, the inventors discovered that the human monocytes died after bridging (or engagement) of the surface molecule APO1/CD95/Fas, and have shown that HGH decreases the death mediated through the molecule

Fas, by increasing the synthesis of an antiapoptogenic proto-oncogene Bcl-2.

Finally, the inventors have studied the effects of HGH on the -TNF response, because Fas and the p55 receptor of the α-TNF belong to the same family of nerve growth receptors. The human promyeloid leukemic line U937 has been used to carry out this work, because of the insensitivity of human monocytes to the death mediated by α-TNF. Obtaining results opposite those observed with Fas, namely that HGH accelerates the death of these cells mediated by α-TNF, has permitted the inventors to conclude as to the inhibitory effect of HGH on the activation of NF-κB by α-TNF, or by other cytotoxic molecules activating NF-κb, such as daunomycin.

Thus, the present invention has for its object to provide a new method for the treatment of cancers, and more particularly malignant hemopathies and solid tumors, offering the advantage of improving both the response of the sick person to certain anti-cancer treatments and also, potentially, the general condition of the sick person.

The invention also has for its object to provide new products for the treatment of said pathologies, having both the advantage of increasing the tumoral cell response to chemotherapy, and to improve the general condition of the patients. The new products of the invention permit decreasing the activation of the NF-κB factor by means of the compound that is used to inhibit the activation of NF-κB, such as the

human growth hormone, which is adapted to give rise to the inhibition of the transcription of the *MDR* genes and hence a reinforcement of the cytotoxic effects of the anti-tumor agents used, with the expected result of decreasing the dosage of these anti-tumor medications.

The invention has for its object the use of compounds inhibiting the activation of NF-κB, for the preparation of medications adapted for the treatment of malign hemopathies and solid tumors.

The invention more particularly has for its object the use of NF-κB inhibitor compounds, for the preparation of medications for the prevention of the appearance or the treatment of phenomena of resistance to cytotoxic molecules used in the field of treatment of the above-mentioned pathologies, these resistance phenomena arising in patients treated with these molecules when these latter are adapted to activate NF-κB.

By compounds inhibiting the activation of NF-κB (also called NF- B inhibitor compounds), there is meant any compound capable of inhibiting in the cells of the organism, the activation of NF-κB caused by the cytotoxic molecules used in the field of treatment of the above-mentioned pathologies, and hence any compound capable of inhibiting the synthesis of proteins (such as P-gp) permitting the cells to eliminate the molecules before they can reach their molecular targets.

The invention relates more particularly to the above-mentioned use of compounds inhibiting the activation of NF-κB, in association with one or several cytotoxic molecules usable in the field

of treatment of malign hemopathies or solid tumors, said cytotoxic molecules being adapted to activate the NF-κB factor.

Preferably, the compounds inhibiting the activation of NF-κB used in the scope of the present invention, are compounds binding specifically to the transmembrane receptors of the cytokines of class I in the cells of the organism. Preferably, said compounds are selected from those binding to the above-mentioned receptors whose amino acid sequences of the transmembrane, intracytoplasmic and extramembrane portions have a homology of about 50% to about 70%.

The invention has more particularly for its object the above-mentioned use of compounds inhibiting the activation of NF-κB as defined above, selected from growth hormone, prolactin, erythropoietin, interleukin-4, interleukin-7, G-CSF, GM-CSF, interleukin-3, interleukin-6, of human or other mammal origin.

Preferably, said compounds are selected from growth hormone or erythropoietin.

In this connection the invention has more particularly for its object the above-mentioned use:

- of human growth hormone, as obtained by extraction from hypophysary extracts, and purification,

- or, preferably, of the recombinant human growth hormone as encoded by the nucleotide SEQ ID NO 1, or by any nucleotide sequence derived from this latter by degeneracy of the genetic code and being nevertheless capable of encoding for the human growth hormone whose

sequence in amino acids is represented by SEQ ID NO 2, said growth hormone being obtained by transformation of suitable cells with the help of vectors containing a nucleotide sequence such as described above, recovery of the recombinant protein produced by said cells, and purification.

The invention also relates to the above-mentioned use, of any peptide sequence derived by addition and/or deletion and/or substitution of one or several amino acids of the sequence SEQ ID NO 2, and conserving the property of the human growth hormone of inhibiting the activation of NF-kB.

The invention has more particularly for its further object the above-mentioned use of recombinant human erythropoietin such as encoded by the nucleotide sequence SEQ ID NO 3, or by any nucleotide sequence derived from this latter by degeneracy of the genetic code and being nevertheless capable of encoding for human erythropoietin, whose sequence in amino acids is represented by SEQ ID NO 4, said erythropoietin being obtained by transformation of appropriate cells with the aid of vectors containing a nucleotide sequence such as described above, recovery of the recombinant protein produced by said cells, and purification.

The invention also relates to the above-mentioned use, of any peptide sequence derived by addition and/or deletion and/or substitution of one or several amino acids of the sequence SEQ ID NO 4, and preserving the property of human erythropoietin of inhibiting the activation of NF-

κB.

The invention has more particularly for its object the above-mentioned use of compounds inhibiting the activation of NF-κB as defined above, for the preparation of a medication administrable by the parenteral route (IM, IV, SC), particularly in the amount of:

- about 2 IU/kg of body weight/day in the case of human growth hormone,

- of about 150 IU/kg of body weight/day in the case of human erythropoietin.

Among the cytotoxic molecules adapted to activate the NF-κB factor used in association with said compounds inhibiting the activation of NF-κB within the scope of the present invention, can be cited:

- the cytokines,

- the anthracyclines, of which may be mentioned daunomycin, and dauxorubicin,

- the vinca-alkaloids, such as vinblastine and vincristin,

- paclitaxel (or Taxol, DCI).

Preferably, the dosage of the cytotoxic molecules used in association with said compounds is about 2 to about 5 times less than the dosage of these same molecules used alone in the scope of the treatment of malignant hemopathies and solid tumors.

By way of illustration:

- the usual daily dose of daunomycin or dauxorubicin being from 40 to 60 mg/m^2, the dosage of these latter in the scope of the

present invention is about 5 to 30 mg/m^2,

- the usual daily dosage of vinblastine being from 5 to 7 mg/m^2, the dosage of this latter in the scope of the present invention is about 1 to 4 mg/m^2,

- the usual daily dosage of vincristin being from 1 to 2 mg/m^2, the dosage of this latter in the scope of the present invention is about 0.1 to 1 mg/m^2,

- the usual daily dosage of taxol being about 75 mg/m^2, the dosage of this latter in the scope of the present invention is about 15 to 35 mg/m^2.

Among the cancers adapted to be treated in the scope of the present invention, can be cited principally:

- malignant hemopathies such as leukemias, lymphomas,

- solid tumors such as those of the ovary or the breast.

The invention also has for its object any product containing:

- a compound inhibiting the activity of NF-κB such as described above, and more particularly a compound binding specifically to the transmembrane receptors of the class I cytokines as defined above,

- and a cytotoxic molecule adapted to activate the NF-κB factor,

as a combined preparation for simultaneous use, separate or prolonged over time, for the treatment of malignant hemopathies and solid tumors.

The invention also has for its object any product as defined

above, as a combined preparation for simultaneous use, separate or over time, for the prevention of the appearance, or for the treatment, of phenomena of resistance to cytotoxic molecules used in the scope of treatment of the above-mentioned pathologies, appearing in patients treated with molecules when these latter are adapted to activate NF-κB.

The invention relates more particularly to any product as defined above, characterized in that it comprises as a compound inhibiting the activation of NF-κB, growth hormone, prolactin, erythropoietin, interleukin-4, interleukin-7, G-CSF, GM-CSF, interleukin-3, interleukin-6.

Products particularly preferred in the scope of the present invention, are those comprising as a compound inhibiting the activation of NF-κB, growth hormone or erythropoietin.

The invention has more particularly for its object any product as defined above, characterized in that it comprises:

- human growth hormone obtained by extraction from hypophysary extracts, and purification,

- or, preferably, recombinant human growth hormone as described above, encoded by the nucleotide sequence SEQ ID NO 1, or by any nucleotide sequence derived from this latter by degeneracy of the genetic code and being nevertheless capable of encoding for the human growth hormone whose sequence of amino acids is represented by SEQ ID NO 2, or any peptide sequence derived by addition and/or deletion and/or substitution of one or several amino acids of the sequence SEQ ID NO 2,

and preserving the property of human growth hormone to inhibit the activation of NF-κB.

The invention also has for its object any product as defined above, characterized in that it comprises recombinant human erythropoietin such as described above, encoded by the nucleotide sequence SEQ ID NO 3, or by any nucleotide sequence derived from this latter by degeneracy of the genetic code and being nevertheless capable of encoding for human erythropoietin whose sequence in amino acids is represented by SEQ ID NO 4, or any peptide sequence derived by addition and/or deletion and/or substitution of one or several amino acids of the sequence SEQ ID NO 4, and preserving the property of human erythropoietin to inhibit the activation of NF-κB.

The invention also relates to any product as described above, characterized in that it comprises as cytotoxic molecule adapted to activate the NF-κB factor, any molecule selected from the following:

- cytokines,
- anthracyclines, such as daunomycin or dauxorubicin,
- vinca-alkaloids, such as vinblastine and vincristine,
- paclitaxel (or Taxol, DCI).

Products such as those defined above that are preferred in the scope of the present invention, are characterized in that they contain:

- growth hormone and daunomycin or dauxorubicin, in proportions such that their daily dosage is about 2 IU/kg of growth

hormone for about 5 to 30 mg/m² of daunomycin or dauxorubicin,

- growth hormone and vinblastine, in proportions such that their daily dosage is about 2 IU/kg of growth hormone for about 1 to 4 mg/m² of vinblastine,

- growth hormone and vincristine, in proportions such that their daily dosage is about 2 IU/kg of growth hormone for about 0.1 to 1 mg/m² of vincristine,

- growth hormone and taxol, in proportions such that their daily dosage is about 2 IU/kg of growth hormone for about 15 to 35 mg/m² of taxol,

- erythropoietin and daunomycin or dauxorubicin, in proportions such that their daily dosage is about 150 IU/kg of erythropoietin for about 5 to 3 mg/m² of daunomycin or dauxorubicin,

- erythropoietin and vinblastine, in proportions such that their daily dosage is about 150 IU/kg of erythropoietin for about 1 to 4 mg/m² of vinblastine,

- erythropoietin and vincristine, in proportions such that their daily dosage is about 150 IU/kg of erythropoietin for about 0.1 to 1 mg/m² of vincristine,

- erythropoietin and taxol, in proportions such that their daily dosage is about 150 IU/kg of erythropoietin for about 15 to 35 mg/m² of taxol.

The invention is illustrated with the help of the following detailed description of the *in vitro* effect of growth hormone and erythropoietin on tumoral cell lines.

1) Example No. 1:

A selection gene (*neomycin resistant*, Neo^R) and the gene encoding for human growth hormone (HGH) have been co-transfected in the human promyeloid leukemic line U937. By comparing the transfected line U937-HGH (which produces in a constituent fashion HGH at physiologic doses), either to the parent line U937, or to a line transfected with Neo^R alone, there are observed by different methodological approaches, that the U937-HGH line dies more under the effect of the *tumor necrosis factor* (-TNF). This cytokine secreted by different types of immune cells has an anti-tumor activity (Harakana, K et al., 1984, Int J Cancer, 34:263-267) and is capable of promoting the activation of NF-κB (Baeuerle PA, Henkel T, 1994, Ann Rev Immunol, 12:141-179).

The U937-HGH cells and the U937-Neo control cells have been cultured for 48 hours in the presence of increasing concentrations of recombinant -TNF. As a result of this culture, the washed cells have been incubated in the presence of propidium iodide which is incorporated in the DNA of the dead cells. These cells are analyzed by flowing cytometry.

Figure 1 shows the increase of the incorporation of propidium iodide as a function of increasing doses of -TNF expressed in international units (IU). For the U937 cells (the mother line having

served to obtain the U937-HGH lines), with increase of the concentration of -TNF, there is observed a slight increase of the percentage of fluorescent cells (thus dead) due to the incorporation of propidium iodide (red fluorescence). This figure shows on the other hand clearly the fact that these values are much higher for the U937-HGH line, as a function of increasing doses of -TNF added to the same culture.

It is thus demonstrated that the presence in the cellular cultures of HGH produced by the U937 lines transfected with the HGH gene, increases their susceptibility to the induction of death mediated by -TNF.

2) Example No. 2:

Having reported in a previous study that HGH could intervene in the inhibition of the activation of NF-κB mediated by lipopolysaccharides (Haeffner A et coll., 1997, J Immunol, 158:1310-1314), the inventors have studied the status of NF-κB during stimulation of the different lines by -TNF.

Figure 2 shows the result of an analysis by gel delay. On this gel were deposited nuclear extracts from the U937-HGH and U937 cells (the mother line having served for obtaining the U937-HGH lines) subjected to different inductors including -TNF or -TNF and cycloheximide (inhibitor of protein synthesis). This experiment indicates clearly that the presence of NF-κB in the nuclei of the U937-HGH cells, is decreased relative to the control cells.

The presence of NF-κB is seen in lines 4 and 5, which

represent the migration of the nuclear extracts of U937 cells stimulated by -TNF, and pre-incubated, either with a cold probe muted NF-κB which does not displace the signal (line 4), or with a cold probe NF-κB homolog which inhibits the signal (line 5).

Figure 3 shows the result of an *enzyme immunoassay* (ELISA) carried out with the lysate of U937-HGH and U937-Neo cells transfected in a transitory manner with a plasmid containing NF-κB sequences in the promotor of the reporter gene encoding for chloramphenicol-acetyl-transferase (CAT) (Chiao P et coll., 1994, Proc Natl Acad Sci USA, 91:28-32).

The cells are transfected by electroporation then incubated with -TNF. At the end of culturing, the cells are lysated and the activated CAT is measured by an commercial ELISA (Boehringer-Mannheim), according to the directions of the supplier.

The figure shows that the CAT activity, reflected by the presence of NF-κB, is decreased in the U937-HGH cells relative to the control cells, after stimulation by -TNF.

The results shown in Figures 2 and 3 therefore show by two different methodological approaches, that the synthesis of NF-κB is decreased in U937-HGH relative to the control line.

3) Example No. 3:

The use of -TNF being very difficult in human clinical work because of the adverse side effects, the inventors are interested in daunomycin. This anthracyclin used in anti-cancer therapy under the name

of Cerubidine acts by insertion in the cellular DNA sequences, thus disturbing the cellular function. Like -TNF (Baeuerle PA, Henkel T, 1994, Ann Rev Immunol, 12:141-179), daunomycin activates NF-κB (Das KC, White CW, 1997, J Biol Chem, 272:14914-14920).

Figure 4 indicates that the U937-HGH line is also more sensitive than the control line to the mediated death by daunomycin.

4) Example 4:

To test the possibility of using the object of the present invention on non-lymphoid tumors, the inventors have used HGH to try to invert the "adriamycine resistant" phenotype of cells isolated from a human ovarian adenocarcinoma IGROV/ADR (Bénard J et coll., 1985, Cancer Res, 45:4970-4979).

As shown by Figure 5, these cells are insensitive to the toxic effect of the daunomycin added to the culture (HGH groups 0 ng/ml). The addition of recombinant HGH (Saizen®, Serono laboratory) renders these cells sensitive to daunomycin, with a maximum effect observed for the lowest dose of HGH used here, namely 5 ml/ml.

These result proves on the one hand that the results of aggravated mortality can be obtained as well with recombinant exogenous HGH as with the transfected lines mentioned above, and that on the other hand, the present invention can be applied to non-lymphoid solid tumors.

5) Example No. 5:

Erythropoietin (EPO), another molecule than HGH belonging to the same family of cytokines of class I, has been tested on human renal

carcinoma cells (RCC) HIEG.

4.10^4 RCC cells have been transfected in a transitory manner with the help of an Effecten^R kit, or with 3μg of plasmid carrying the gene encoding for EPO (RCC-EPO cells), or with 3μg of a plasmid coding for the resistance to neomycin (RCC-Neo cells) as the negative control. After 48 hours, the RCC were combined with daunomycin at two different concentrations: 0.3 and 0.6 μM. The number of surviving cells was measured 48 hours later by flow cytometry (Figure 6).

The results of Experiment 1 expressed in numbers of living cells are as follows:

	RCC-Neo	RCC-EPO
daunomycin 0μM	14745	26911
daunomycin 0.3μM	11382	3487
daunomycin 0.6μM	10179	8551

The results of Experiment 2 expressed in numbers of living cells are as follows:

	RCC-Neo	RCC-EPO
daunomycin 0μM	20150	29102
daunomycin 0.3μM	8891	2693
daunomycin 0.6μM	7001	4739

The results show that in the two different experiments (Experiments 1 and 2), the conjoint presence of daunomycin and EPO aggravates substantially the cellular mortality, with a more marked effect for the lower dose of daunomycin used.

DESCRIPTION OF THE DRAWINGS

- Figure 1: The effect of growth hormone on the mortality of cells exposed to -TNF: the percentage of the dead cells (IP+) is indicated on the ordinate, the white colonies corresponding to the cells of the strain U937-Neo, the black colonies corresponding to the cells of the strain U937-hGH; the concentrations of NFT- are indicated on the abscissa in IU/ml.

- Figure 2: The effect of growth hormone on the translocation of NF-κB; column 1 corresponds to the control cells U937, column 2 corresponds to the U937 cells treated with -TNF + cycloheximide, column 3 corresponds to the U937 cells treated with -TNF, column 4 corresponds to the U937 cells treated with -TNF + a mutant NF-κB probe, column 5 corresponds to the U937 cells treated with -TNF + a homologous NF-κB probe, column 6 corresponds to the control cells U937-HGH, column 7 corresponds to the U937-HGH cells treated with -TNF + cycloheximide, column 8 corresponds to the U937-HGH cells treated with -TNF; the presence of NF-κB is indicated by an arrow.

- Figure 3: Effect of growth hormone on the reporter activity CAT; the percentage of variation of CAT activity is indicated on the abscissa; the two left columns show the two experiments carried out on U937-Neo cells, and the two right columns represent the two independent experiments carried out on U937-HGH cells.

- Figure 4: Effect of growth hormone on apoptosis induced by daunomycin; the percentage of the dead cells (IP+) is indicated on the

ordinate, the white columns corresponding to the cells of the strain U937-Neo, the black columns corresponding to the cells of the strain U937-HGH; the indicated percentages show the increase of mortality of the cells; the concentrations of daunomycin are indicated on the abscissa in μM.

- Figure 5: Effect of growth hormone on the apoptosis of the IGROV/ADR line, induced by daunomycin; the percentage of dead cells (IP+) is indicated on the ordinate, and different columns corresponding to the different concentrations of HGH used (0, 5, 50, 500, 1000 ng/ml); the concentrations of daunomycin are indicated on the abscissa in μM.

- Figure 6: Effect of erythropoietin on the apoptosis of the human renal carcinoma line HIEG, induced by daunomycin: for each of the experiments 1 and 2, the number of living cells is indicated on the ordinate, the white columns correspond to the RCC-Neo cells, the black columns correspond to the RCC-EPO cells; the concentrations of daunomycin are indicated on the abscissa in μM.

What is claimed is:

1. The use of compounds inhibiting the activation of the nuclear factor κB (NF-κB), for the preparation of medications adapted for the treatment of malignant hemopathies and solid tumors, and for the prevention of the appearance or the treatment, of phenomena of resistance to cytotoxic molecules used in the scope of treatment of the above pathologies, appearing in patients treated with these molecules when the latter are adapted to activate NF-κB.

2. The use of inhibitor compounds for the activation of NF-κB according to claim 1, for the preparation of medications adapted for the treatment of malignant hemopathies and solid tumors, in combination with one or several cytotoxic molecules usable in the scope of treatment of the above-mentioned pathologies and adapted to activate the NF-κB factor.

3. The use according to claim 1 or claim 2, of compounds inhibiting the activation of MF-κB connected specifically to the transmembranal receptors of the cytokins of class I in the cells of the organism, such as compounds selected from growth hormone or erythropoietin.

4. The use according to one of claims 1 to 3:

- of the human growth hormone, as obtained by extraction from hypophysary extracts, and purification,

- or of the recombinant human growth hormone as encoded by the nucleotide sequence SEQ ID NO 1, or by any nucleotide sequence derived from this latter by degeneracy of the genetic code and being

nevertheless capable of encoding for the human growth hormone whose sequence in amino acids is represented by SEQ ID NO 2, said growth hormone being obtained by transformation of appropriate cells with the help of vectors containing a nucleotide sequence as described, recovery of the recombinant protein produced by said cells, and purification,

- or of any peptide sequence derived by addition and/or deletion and/or substitution of one or several amino acids of the sequence SEQ ID NO 2,

and preserving the property of human growth hormone of inhibiting the activation of NF-κB.

5. The use according to oneof claims 1 to 3:

- of recombinant human erythropoietin such as encoded by the nucleotide sequence SEQ ID NO 3, or by any nucleotide sequence derived from this latter by degeneracy of the genetic code and being nevertheless capable of encoding for human erythropoietin whose sequence in amino acids is represented by SEQ ID NO 4, said erythropoietin being obtained by transformation of appropriate cells with the help of vectors contained in a nucleotide sequence as described above, recovery of the recombinant protein produced by said cells, and purification,

- or any peptide sequence derived by addition and/or deletion and/or substitution of one or several amino acids of the sequence SEQ ID NO 4, and preserving the property of inhibiting the activation of NF-κB.

6. The use compounds inhibiting the activation of NF-κB according to one of claims 1 to 7, in combination with one or several

cytotoxic molecules adapted to activate the NF-κB factor, selected from:

- cytokines,
- anthracyclines, including daunomycin, and dauxorubicin,
- vinca-alkaloids, such as vinblastine and vincristine,
- paclitaxel (or Taxel, DCI).

7. The use of compounds inhibiting the activation of NF-κB according to one of claims 1-6, characterized in that the dosage of the cytotoxic molecules used in combination with said compounds is about 2 to about 5 times less than the dosage of these same molecules used alone in the scope of treatment of malignant hemopathies and solid tumors.

8. Products containing a compound inhibiting the activation of NF-κB and a cytotoxic molecule adapted to activate the NF-?B factor, as a combined preparation for a simultaneous use, separately or over a long period of time for the treatment of malignant hemopathies and solid tumors.

9. Product according to claim 8, characterized in that it comprises as a compound inhibiting the activation of NF-κB, a compound specifically binding to class I cytokine transmembrane receptors in the cells of the organism, selected particularly from growth hormone or erythropoietin.

10. Product according to claim 8 or 9, characterized in that it comprises:

- human growth hormone, such as obtained by the extraction from hypophysary extracts, and purification,

- or recombinant human growth hormone as encoded by the nucleotide sequence SEQ ID NO 1, or by any nucleotide sequence derived from this latter by degeneracy of the genetic code and being nevertheless capable of encoding for human growth hormone whose amino acid sequence is represented by SEQ ID NO 2, said growth hormone being obtained by a transformation of suitable cells with the help of vectors containing a nucleotide sequence such as described above, recovery of the recombinant protein produced by said cells, and purification,

- or any peptide sequence derived by addition and/or deletion and/or substitution of one or several amino acids of the sequence SEQ ID NO 2, and keeping the property of the human growth hormone of inhibiting the activation of NF-κB.

11. Product according to claim 8 or 9, characterized in that it comprises:

- recombinant human erythropoietin as encoded by the nucleotide sequence SEQ ID NO 3, or by any nucleotide sequence derived from this latter by degeneracy of the genetic code and being nevertheless capable of encoding for human erythropoietin whose sequence in amino acids is represented by SEQ ID NO 4, said erythropoietin being obtained by transformation of suitable cells with the help of vectors containing a nucleotide sequence as described above, recovery of the recombinant protein produced by said cells, and purification,

- or any peptide sequence derived by addition and/or deletion and/or substitution of one or several amino acids of the sequence SEQ

ID NO 4, and keeping the property of human erythropoietin of inhibiting the activation of NF-κB.

12. Product according to one of claims 8 to 11, characterized in that it comprises as cytotoxic molecule susceptible of activating the NF-κB factor, any molecule selected from the following:

- cytokines,
- anthracyclines including daunomycin and dauxorubicin,
- vinca-alkaloids, including vinblastine and vincristine,
- paclitaxel (or Taxol, DCI).

ABSTRACT OF THE DISCLOSURE

Compounds inhibiting the activation of the nuclear factor κB (NF-κB) are used for the preparation of medications adapted for the treatment of malignant hemopathies and solid tumors, and for the prevention of the appearance or the treatment, of phenomena of resistance to cytotoxic molecules used in the scope of treatment of the above pathologies, appearing in patients treated with these molecules when the latter are adapted to activate NF-κB.

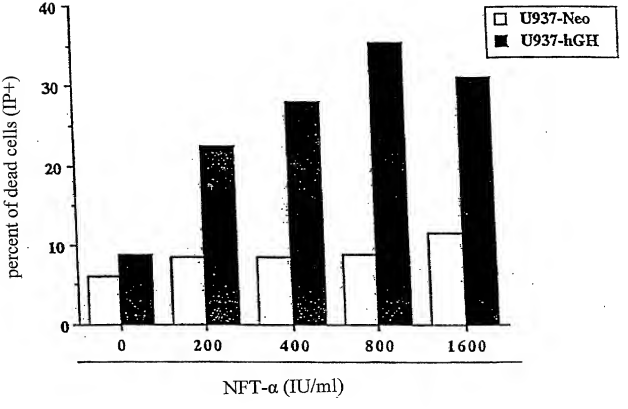

40
30
20
10
0
percent of dead cells (IP+)
U937-Neo
U937-hGH
0
200
400
800
1600
NFT-α (IU/ml)

FIGURE 1

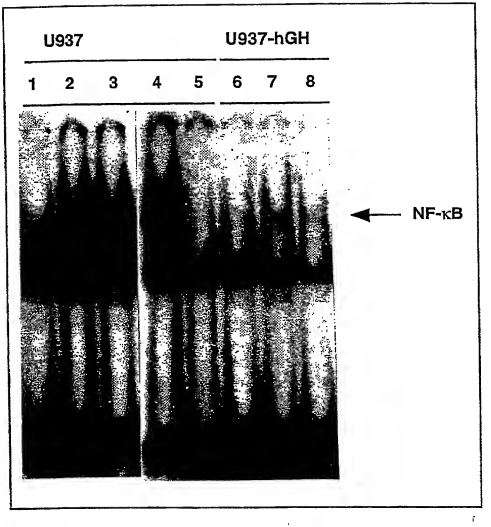


FIGURE 2

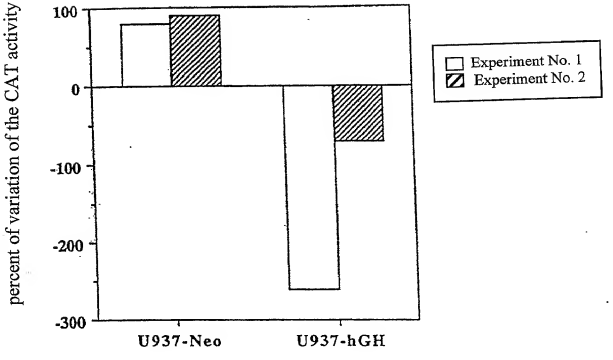

percent of variation of the CAT activity
100
0
-100
-200
-300
U937-Neo
U937-hGH
Experiment No. 1
Experiment No. 2

FIGURE 3

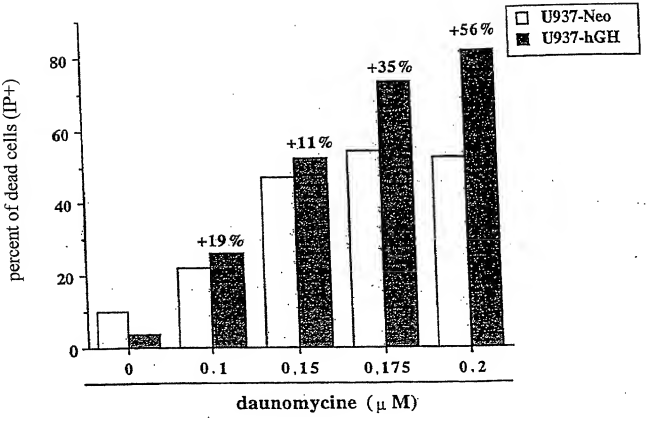

U937-Neo
U937-hGH
+56%
+35%
+11%
+19%
80
60
40
20
0
percent of dead cells (IP+)
0
0.1
0.15
0.175
0.2
daunomycine (μ M)

FIGURE 4

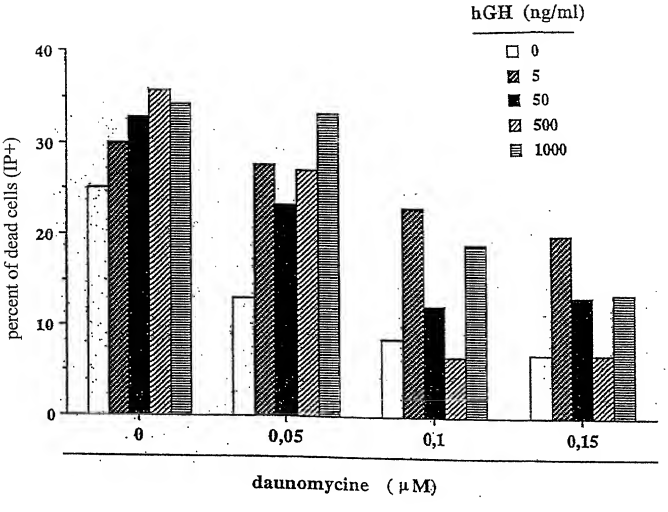
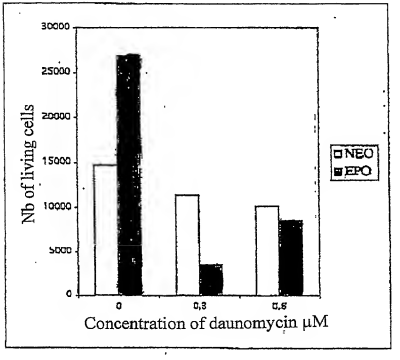


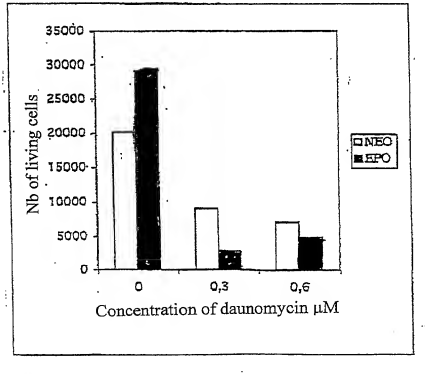
FIGURE 5

Figure 6

Experiment 1



Experiment 2



COMBINED DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

NF-KB ACTIVATION INHIBITORS, AND THEIR PHARMACEUTICAL USES

the specification of which: *(check one)*

REGULAR OR DESIGN APPLICATION

[ ] is attached hereto.

[ ] was filed on as application Serial No. and was amended on (if applicable).

PCT FILED APPLICATION ENTERING NATIONAL STAGE

[X] was described and claimed in International application PCT/FR99/02897 filed on 24 November 1999 and as amended on (if any).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

PRIORITY CLAIM

I hereby claim foreign priority benefits under 35 USC 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed.

PRIOR FOREIGN APPLICATION(S)

Country	Application Number	Date of Filing (day, month, year)	Priority Claimed
France	**98/14858**	**25 November 1998**	**yes**

(Complete this part only if this is a continuing application.)

I hereby claim the benefit under 35 USC 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of 35 USC 112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37 Code of Federal Regulations §1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application:

(Application Serial No.) (Filing Date) (Status--patented, pending, abandoned)

POWER OF ATTORNEY

The undersigned hereby authorizes the U.S. attorney or agent named herein to accept and follow instructions from **Grosset-Fournier & Demachy s.a.r.l.** as to any action to be taken in the Patent and Trademark Office regarding this application without direct communication between the U.S. attorney or agent and the undersigned. In the event of a change in the persons from whom instructions may be taken, the U.S. attorney or agent named herein will be so notified by the undersigned.

As a named inventor, I hereby appoint the registered patent attorneys represented by Customer No. **000466** to prosecute this application and transact all business in the Patent and Trademark Office connected therewith, including: **Robert J. PATCH, Reg. No. 17,355, Andrew J. PATCH, Reg. No. 32,925, Robert F. HARGEST, Reg. No. 25,590, Benoît CASTEL, Reg. No. 35,041, Eric JENSEN, Reg. No. 37,855, Thomas W. PERKINS, Reg. No. 33,027, and Roland E. LONG, Jr., Reg. No. 41,949,**

c/o YOUNG & THOMPSON,
Second Floor,
745 South 23rd Street,
Arlington, Virginia 22202.

00466
PATENT TRADEMARK OFFICE

Address all telephone calls to Young & Thompson at 703/521-2297. Telefax: 703/685-0573.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full name of sole or first inventor: **Francois HIRSCH**
(given name, family name)

Inventor's signature _____ Date May 28th, 2001

Residence: **Arcueil, France** FRX Citizenship: **French**

Post Office Address: 20, rue Victor Carmignac
F-94110 Arcueil, France

Full name of second joint inventor, if any: **Astrid HAEFFNER**
(given name, family name)

Inventor's signature _____ Date 28 Mai 2001

Residence: **Meudon La Foret, France** FRX Citizenship: **French**

Post Office Address: **14, avenue de Celles**
F-92360 Meudon La Foret, France

LIST OF SEQUENCES

(1) GENERAL INFORMATION:

(i) DEPOSITOR:
(A) NAME: CENTRE NATIONAL DE LA RECHERCHE SCIENTIFIQUE
(B) STREET: 3, rue Michel-Ange
(C) CITY: PARIS
(E) COUNTRY: FRANCE
(F) POSTAL CODE: 75794 CEDEX 16

(ii) TITLE OF THE INVENTION: NF-κB ACTIVATION INHIBITORS, AND THEIR PHARMACEUTICAL USES

(iii) NUMBER OF SEQUENCES: 4

(iv) COMPUTER READABLE FORM:
(A) TYPE OF SUPPORT: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) USER SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (OEB)

(2) INFORMATION FOR THE SEQ ID NO: 1:

(i) CHARACTERISTICS OF THE SEQUENCE:
(A) LENGTH: 609 base pairs
(B) TYPE: nucleotide
(C) NUMBER OF STRANDS: double
(D) CONFIGURATION: linear

(ii) TYPE OF MOLECULE: ADN (g enomic)

(ix) CHARACTERISTIC:
(A) NAME/KEY: CDS
(B) EMPLACEMENT:1..609

(xi) DESCRIPTION OF THE SEQUENCE: SEQ ID NO: 1:

```
ATG GCT ACA GGC TCC CGG ACG TCC CTG CTC CTG GCT TTT GGC CTG CTC        48
Met Ala Thr Gly Ser Arg Thr Ser Leu Leu Leu Ala Phe Gly Leu Leu
 1               5                  10                  15

TGC CTG CCC TGG CTT CAA GAG GGC AGT GCC TTC CCA ACC ATT CCC TTA        96
Cys Leu Pro Trp Leu Gln Glu Gly Ser Ala Phe Pro Thr Ile Pro Leu
            20                  25                  30

TCC AGG CTT TTT GAC AAC GCT AGT CTC CGC GCC CAT CGT CTG CAC CAG       144
Ser Arg Leu Phe Asp Asn Ala Ser Leu Arg Ala His Arg Leu His Gln
        35                  40                  45
```

```
CTG GCC TTT GAC ACC TAC CAG GAG TTT AAC CCC CAG ACC TCC CTC TGT      192
Leu Ala Phe Asp Thr Tyr Gln Glu Phe Asn Pro Gln Thr Ser Leu Cys
     50                  55                  60

TTC TCA GAG TCT ATT CCG ACA CCC TCC AAC AGG GAG GAA ACA CAA CAG      240
Phe Ser Glu Ser Ile Pro Thr Pro Ser Asn Arg Glu Glu Thr Gln Gln
 65                  70                  75                  80

AAA TCC AAC CTA GAG CTG CTC CGC ATC TCC CTG CTG CTC ATC CAG TCG      288
Lys Ser Asn Leu Glu Leu Leu Arg Ile Ser Leu Leu Leu Ile Gln Ser
                 85                  90                  95

TGG CTG GAG CCC GTG CAG TTC CTC AGG AGT GTC TTC GCC AAC AGC CTG      336
Trp Leu Glu Pro Val Gln Phe Leu Arg Ser Val Phe Ala Asn Ser Leu
            100                 105                 110

GTG TAC GGC GCC TCT GAC AGC AAC GTC TAT GAC CTC CTA AAG GAC CTA      384
Val Tyr Gly Ala Ser Asp Ser Asn Val Tyr Asp Leu Leu Lys Asp Leu
        115                 120                 125

GAG GAA GGC ATC CAA ACG CTG ATG GGG AGG CTG GAA GAT GGC AGC CCC      432
Glu Glu Gly Ile Gln Thr Leu Met Gly Arg Leu Glu Asp Gly Ser Pro
    130                 135                 140

CGG ACT GGG CAG ATC TTC AAG CAG ACC TAC AGC AAG TTC GAC ACA AAC      480
Arg Thr Gly Gln Ile Phe Lys Gln Thr Tyr Ser Lys Phe Asp Thr Asn
145                 150                 155                 160

TCA CAC AAC GAT GAC GCA CTA CTC AAG AAC TAC GGG CTG CTC TAC TGC      528
Ser His Asn Asp Asp Ala Leu Leu Lys Asn Tyr Gly Leu Leu Tyr Cys
                165                 170                 175

TTC AGG AAG GAC ATG GAC AAG GTC GAG ACA TTC CTG CGC ATC GTG CAG      576
Phe Arg Lys Asp Met Asp Lys Val Glu Thr Phe Leu Arg Ile Val Gln
            180                 185                 190

TGC CGC TCT GTG GAG GGC AGC TGT GGC TTC TAG                          609
Cys Arg Ser Val Glu Gly Ser Cys Gly Phe  *
        195                 200
```

(2) INFORMATION FOR THE SEQ ID NO: 2:

(i) CHARACTERISTICS OF THE SEQUENCE:
(A) LENGTH: 203 amino acids
(B) TYPE: amino acid
(D) CONFIGURATION: linear

(ii) MOLECULE TYPE: protein
(xi) DESCRIPTION OF THE SEQUENCE: SEQ ID NO: 2:

```
Met Ala Thr Gly Ser Arg Thr Ser Leu Leu Leu Ala Phe Gly Leu Leu
 1               5                  10                  15
```

```
Cys Leu Pro Trp Leu Gln Glu Gly Ser Ala Phe Pro Thr Ile Pro Leu
                20                  25                  30

Ser Arg Leu Phe Asp Asn Ala Ser Leu Arg Ala His Arg Leu His Gln
            35                  40                  45

Leu Ala Phe Asp Thr Tyr Gln Glu Phe Asn Pro Gln Thr Ser Leu Cys
        50                  55                  60

Phe Ser Glu Ser Ile Pro Thr Pro Ser Asn Arg Glu Glu Thr Gln Gln
65                  70                  75                  80

Lys Ser Asn Leu Glu Leu Leu Arg Ile Ser Leu Leu Leu Ile Gln Ser
                85                  90                  95

Trp Leu Glu Pro Val Gln Phe Leu Arg Ser Val Phe Ala Asn Ser Leu
            100                 105                 110

Val Tyr Gly Ala Ser Asp Ser Asn Val Tyr Asp Leu Leu Lys Asp Leu
        115                 120                 125

Glu Glu Gly Ile Gln Thr Leu Met Gly Arg Leu Glu Asp Gly Ser Pro
    130                 135                 140

Arg Thr Gly Gln Ile Phe Lys Gln Thr Tyr Ser Lys Phe Asp Thr Asn
145                 150                 155                 160

Ser His Asn Asp Asp Ala Leu Leu Lys Asn Tyr Gly Leu Leu Tyr Cys
                165                 170                 175

Phe Arg Lys Asp Met Asp Lys Val Glu Thr Phe Leu Arg Ile Val Gln
            180                 185                 190

Cys Arg Ser Val Glu Gly Ser Cys Gly Phe  *
        195                 200
```

(2) INFORMATION FOR THE SEQ ID NO: 3:

(i) CHARACTERISTICS OF THE SEQUENCE:
(A) LENGTH: 582 base pairs
(B) TYPE: nucleotide
(C) NUMBER OF STRANDS: double
(D) CONFIGURATION: linear

(ii) MOLECULE TYPE: ADN (genomic)

(ix) CHARACTERISTIC:
(A) NAME/KEY: CDS
(B) EMPLACEMENT:1..582

(xi) DESCRIPTION OF THE SEQUENCE: SEQ ID NO: 3:

```
ATG GGG GTG CAC GAA TGT CCT GCC TGG CTG TGG CTT CTC CTG TCC CTG        48
Met Gly Val His Glu Cys Pro Ala Trp Leu Trp Leu Leu Leu Ser Leu
    205                 210                 215

CTG TCG CTC CCT CTG GGC CTC CCA GTC CTG GGC GCC CCA CCA CGC CTC        96
Leu Ser Leu Pro Leu Gly Leu Pro Val Leu Gly Ala Pro Pro Arg Leu
220                 225                 230                 235

ATC TGT GAC AGC CGA GTC CTG GAG AGG TAC CTC TTG GAG GCC AAG GAG       144
Ile Cys Asp Ser Arg Val Leu Glu Arg Tyr Leu Leu Glu Ala Lys Glu
                240                 245                 250

GCC GAG AAT ATC ACG ACG GGC TGT GCT GAA CAC TGC AGC TTG AAT GAG       192
Ala Glu Asn Ile Thr Thr Gly Cys Ala Glu His Cys Ser Leu Asn Glu
            255                 260                 265

AAT ATC ACT GTC CCA GAC ACC AAA GTT AAT TTC TAT GCC TGG AAG AGG       240
Asn Ile Thr Val Pro Asp Thr Lys Val Asn Phe Tyr Ala Trp Lys Arg
        270                 275                 280

ATG GAG GTC GGG CAG CAG GCC GTA GAA GTC TGG CAG GGC CTG GCC CTG       288
Met Glu Val Gly Gln Gln Ala Val Glu Val Trp Gln Gly Leu Ala Leu
    285                 290                 295

CTG TCG GAA GCT GTC CTG CGG GGC CAG GCC CTG TTG GTC AAC TCT TCC       336
Leu Ser Glu Ala Val Leu Arg Gly Gln Ala Leu Leu Val Asn Ser Ser
300                 305                 310                 315

CAG CCG TGG GAG CCC CTG CAG CTG CAT GTG GAT AAA GCC GTC AGT GGC       384
Gln Pro Trp Glu Pro Leu Gln Leu His Val Asp Lys Ala Val Ser Gly
                320                 325                 330

CTT CGC AGC CTC ACC ACT CTG CTT CGG GCT CTG GGA GCC CAG AAG GAA       432
Leu Arg Ser Leu Thr Thr Leu Leu Arg Ala Leu Gly Ala Gln Lys Glu
            335                 340                 345

GCC ATC TCC CCT CCA GAT GCG GCC TCA GCT GCT CCA CTC CGA ACA ATC       480
Ala Ile Ser Pro Pro Asp Ala Ala Ser Ala Ala Pro Leu Arg Thr Ile
        350                 355                 360

ACT GCT GAC ACT TTC CGC AAA CTC TTC CGA GTC TAC TCC AAT TTC CTC       528
Thr Ala Asp Thr Phe Arg Lys Leu Phe Arg Val Tyr Ser Asn Phe Leu
    365                 370                 375

CGG GGA AAG CTG AAG CTG TAC ACA GGG GAG GCC TGC AGG ACA GGG GAC       576
Arg Gly Lys Leu Lys Leu Tyr Thr Gly Glu Ala Cys Arg Thr Gly Asp
380                 385                 390                 395

AGA TGA                                                               582
Arg  *
```

(2) INFORMATION FOR THE SEQ ID NO: 4:

(i) CHARACTERISTICS OF THE SEQUENCE:
(A) LENGTH: 194 amino acids
(B) TYPE: amino acid
(D) CONFIGURATION: linear

(ii) TYPE OF MOLECULE: protein
(xi) DESCRIPTION OF THE SEQUENCE: SEQ ID NO: 4:

```
Met Gly Val His Glu Cys Pro Ala Trp Leu Trp Leu Leu Leu Ser Leu
 1               5                  10                  15

Leu Ser Leu Pro Leu Gly Leu Pro Val Leu Gly Ala Pro Pro Arg Leu
            20                  25                  30

Ile Cys Asp Ser Arg Val Leu Glu Arg Tyr Leu Leu Glu Ala Lys Glu
        35                  40                  45

Ala Glu Asn Ile Thr Thr Gly Cys Ala Glu His Cys Ser Leu Asn Glu
    50                  55                  60

Asn Ile Thr Val Pro Asp Thr Lys Val Asn Phe Tyr Ala Trp Lys Arg
65                  70                  75                  80

Met Glu Val Gly Gln Gln Ala Val Glu Val Trp Gln Gly Leu Ala Leu
                85                  90                  95

Leu Ser Glu Ala Val Leu Arg Gly Gln Ala Leu Leu Val Asn Ser Ser
            100                 105                 110

Gln Pro Trp Glu Pro Leu Gln Leu His Val Asp Lys Ala Val Ser Gly
        115                 120                 125

Leu Arg Ser Leu Thr Thr Leu Leu Arg Ala Leu Gly Ala Gln Lys Glu
    130                 135                 140

Ala Ile Ser Pro Pro Asp Ala Ala Ser Ala Ala Pro Leu Arg Thr Ile
145                 150                 155                 160

Thr Ala Asp Thr Phe Arg Lys Leu Phe Arg Val Tyr Ser Asn Phe Leu
                165                 170                 175

Arg Gly Lys Leu Lys Leu Tyr Thr Gly Glu Ala Cys Arg Thr Gly Asp
            180                 185                 190

Arg *
```